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Moving beyond Mass: The Unmet Need to Consider Dose Metrics in Environmental Nanotoxicology Studies

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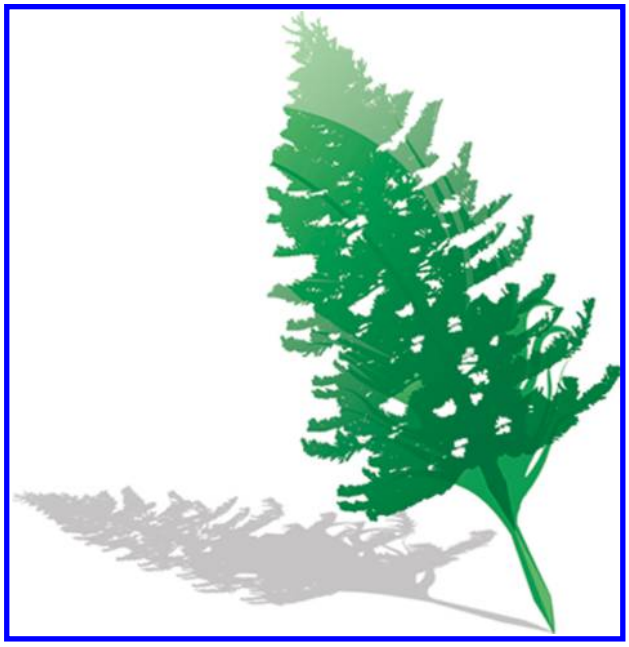

The field of nanotechnology has rapidly developed due to recognition that nanoscale particles often exhibit properties that differ substantially from comparable bulk phases. Given this fact it is unfortunate that the environmental nanotoxicology community continues to rely heavily upon mass-based dose-metrics. To truly evaluate if nanoscale particles elicit toxicological responses that differ from those of bulk phases then alternative dose-metrics must be simultaneously considered.

Despite previous calls to report characterization information sufficient to evaluate the toxicity of engineered nanomaterials against dose-metrics other than mass,[1] most environmental nanotoxicology studies continue to report dosimetry exclusively on a mass basis. We sampled 25 representative studies (most of which were published in 2010−2011) investigating the ecotoxicological effects of unbound engineered nanomaterials. All reported dosimetry exclusively in terms of mass, and less than 20% reported characterization information sufficient to transform mass concentrations to particle number, particle number density (N, particles per unit volume), or suspension surface area. Transforming from mass concentration requires knowledge of the particle size, shape, and crystallinity, which were reported in 60%, 48%, and 20% of the manuscripts, respectively. Only 8% of the studies reported that characterization was performed in the experimental media used to measure a toxicological response and none reported the fraction of dissolved ions present.

Focused efforts to relate dosimetry to biological effects are required to better understand the ecotoxicological profiles of engineered nanomaterials and to allow risk assessors to more reliably relate exposure to effect. We challenge the research community to better address the dosimetry issue in future toxicological studies with engineered nanomaterials, and to report characterization information sufficient to convert mass-based concentration data to other dose-metrics.

When Mass Misleads. The reliance of nanoparticle environmental risk studies in aqueous systems on mass-based dose-metrics is likely attributable to (i) convention—most water quality standards are presented in terms of mass concentration, (ii) ease of measurement, and (iii) an inherent need to compare toxicity of particulate versus dissolved species to delineate potential "nano" effects. The principal assumption behind mass concentration, that the molar mass of a solute is equivalent to 6.022×10^{23} "particles" per mole, is violated at the nanoscale. At a given mass, decreasing particle size exponentially increases particle number, and suspension surface area (by several orders of magnitude) available to interact with biological receptors.[2] Differences in particle number or surface area can dictate a toxicological response and thus the indiscriminate application of mass-only dosimetry discards critical information that may determine toxicity, in particular, the degree to which receptors are saturated with nanoparticles.

Lessons Learned from Aerosol Science and Immune System Responses: Number and Surface Area Matter. The importance of dosimetry in the aerosolized particulate literature is well established. In a study investigating the effects of nanoscale particles on rat alveolar macrophages, Oberdörster[3] reported that surface area concentrations correlate better to inflammatory response and lung tumor incidence than does mass concentration. Donaldson et al.[4] suggested that increasing

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particle number and surface area may overwhelm macrophage defenses and enhance interfacial interactions between reactive particle surfaces and epithelial cells, leading to an increase in the incidence of inflammation and oxidative damage within the lung. In these instances where the mechanism of toxicity is driven primarily by particle number and available surface area, traditional mass-based approaches to dosimetry may misrepresent dose−response relationships. These observations presumably apply to aquatic toxicity studies as well, but the current emphasis on mass-based dosimetry coupled with limited characterization data preclude our ability to confirm this hypothesis.

Determining Particle Number and Suspension Surface Area in Fluid Media. Most particle measurement techniques provide information regarding their mass concentration averages. Other techniques such as optical/electron microscopy and particle counting techniques can provide direct measures of particle number, and particle number density (N), but these techniques are typically much less representative of the whole sample and more labor intensive. For the former, N is calculated through particle dimensionality and particle mass concentration, which can be accurately measured using either elemental concentration or optical turbidity data. For particles in fluid media, surface area can be measured experimentally using a dye adsorption technique, assuming the particles of interest represent the dominant colloids in the system. In this case, the suspension surface area is calculated from the surface area of the dye molecule and the Langmuir sorption maximum, assuming input dye concentrations are insufficient for formation of dimers, trimers, etc.

Mass Still Matters: The Importance of Characterization on Dose-Metric Conversion. In aqueous media, mass concentration is the only dose metric routinely measured using existing analytical techniques. Measures of nanoparticle number and suspension surface area in fluid media are approximations derived from a combination of an accurate measure of mass concentration, particle geometry, and particle size distributions. To facilitate conversions from mass to other dose-metrics, researchers should provide, at a minimum, (i) mass concentration, (ii) primary particle dimensions and shapes based on electron microscopy, (iii) crystallinity, and (iv) understanding of the dissolution characteristics of the material. A nonexhaustive list of example dose-metric extrapolations is provided in Table 1. Researchers should be mindful, however, that many factors other than dosimetry, such as reactive surface area and particle composition, will influence nanoparticle toxicity. Consideration of these factors is essential when interpreting the results of toxicological studies with engineered nanomaterials, but are beyond the scope of the dosimetry issues discussed here.

Until further studies are performed to evaluate the role of nanoparticle dosimetry in hazard evaluations of nanoscale materials, the significance of this current oversight in the nanotechnology risk literature will remain unclear. Although many high quality nanotoxicology studies have been published, far too few of these studies have significant potential to impact nanotoxicology risk decisions due to the lack of nanomaterial characterization and the improper reliance on mass-based dosimetry alone. The nanotoxicology research community must transition to studies that rapidly facilitate development of an improved understanding of how particle size, particle number, and surface area collectively affect dose−response relationships.

Table 1. List of Some Dose Metrics to Consider, Particle Characterization Information Needed (Required and Optional), And an Example Calculation to Transform Mass Measurements to Alternative Dose-Metrics[a]

Mass	
Required: Elemental concentrations (ICP-MS, total organic carbon, etc.), gravimetric, optical turbidity	**Measured Parameter**
Optional: Temporal stability in test media	
Dissolved Fraction (D_f)	
Required: Mass (as above) + diafiltration with low MW cut-off filter or ultracentrifugation	$D_f = \frac{C_d}{C_t}$
Optional: Dissolution kinetics, ion-specific electrodes, voltammetry	
Particle Number and Particle Number Density (N)	
Required: Mass (as above) + experimental particle size and/or particle size distribution (optical/centrifugal/acoustic techniques) + shape (EM) + crystalline structure if multiple structures exist (XRD) + particle/bulk density[b]	$N = \frac{c_t}{V_{NP}} \cdot \frac{V_{Molar}}{MW}$
Optional: UV-Vis spectroscopy for noble metallic nanoparticles	
** If only a single crystal structure exists, $\frac{V_{Molar}}{MW}$ can be replaced by $\frac{1}{\rho_b}$.*	$V_{Molar} = \frac{V_{unitcell}}{Atoms_{UC}} Ag\#$
Total Surface Area (TSA)	
Required: Mass (as above) + primary particle size and shape (EM) + particle number (as above)	$TSA = N * 4\pi R^2$
Optional: Surface area[c] (e.g., dye-adsorption method), particle size (EM, DLS)	***for spherical particles**

[a]Where, D_f = dissolved fraction, c_d = dissolved mass concentration, c_t = total measurable mass concentration, N = particle number, TSA = total surface area, ρ_b = bulk density; R = particle or aggregate radius; MW = molecular weight; V_{Molar} = molar volume; V_{NP} = volume of nanoparticle; V_{uc} = volume of a unit cell (uc); $Atoms_{UC}$ = no. of atoms in a unit cell; Ag# = Avogadro's number. Values for V_{Molar}, $V_{unitcell}$, $Atoms_{UC}$ can be found in the Handbook of Chemistry and Physics. [b]For compounds having multiple crystal structures (such as TiO_2 − rutile, anatase, brookite) knowledge of the crystal structure is required to convert mass to particle number dose-metrics. Disregarding crystal structure can contribute up to 9% error for TiO_2 nanomaterials. [c]Calculated from particle size and morphology.

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Notes

The authors declare no competing financial interest.

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